ORIGINAL RESEARCH PAPER

Volume-7 | Issue-10 | October-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

"ASSESSMENT OF RECENT HEMATOLOGICAL PARAMETERS IN DIAGNOSIS AND FOLLOW UP OF CHRONIC RENAL DISEASES"

Pathology

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KEYWORDS

INTRODUCTION

Despite the fact that automation is indispensible in view of accuracy and efficacy, the manual age old CBC methodology is still functional due to economic considerations and non availability of Automated Cell Counters (ACCs), particularly in the smaller laboratory in developing countries. In 1940s, Wallace Coulter developed a blood cell analysis tool for quick screening of large number of blood samples.[1] Modern ACCs work on light scatter or impedance Coulter principles. In 1968, Dittrich and Goehde demonstrated fluorescence based cytometry by coupling laser beam to flow device discovered by Fulwyer et al.[2] A 5-part differential ACC counts leucocytes, enumerate percentage and absolute number of IGs using a combined technology of cytochemistry, focused flow impedance, and light absorbance. The ongoing improvements in these instruments resulted in enumeration & evaluation of blood cells with precision, speed, more accuracy and at reduced cost.

The "Complete Blood Count" (CBCs) mainly includes Red Blood Cells (RBCs), Hemoglobin (Hb) concentration, White Blood Cells (WBCs), Platelet count and Mean Corpuscular Volume (MCV) from which parameters like hematocrit (Hct), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH) and Red cell Distribution Width (RDW) are derived. Newer ACCs like Sysmex XT-4000i and Advia® 2120i additionally generate immature reticulocyte fraction (IRF), mean reticulocyte volume (MCVr), mean reticulocyte hemoglobin content (CHr), fragmented RBC (FRBC) count and the immature platelet fraction (IPF) parameters.

The reticulocyte count reflects the effective erythropoiesis. The reticulocytes generally circulate in the peripheral blood for 1-2 days after their release from bone marrow. An increased reticulocyte count results from acute bleeding, post-hemolysis, chronic renal disease and response to therapy.[3] A decreased reticulocyte count results from nutritional deficiency anemia and decreased erythropoietin level (chronic renal failure) etc.[3] The Immature Reticulocyte Fraction (IRF) is the quantitative proportion of all younger reticulocytes and is derived as a ratio of immature reticulocytes to the total number of reticulocytes. IRF is a highly sensitive and an early marker of marrow erythropoietic activity and along with reticulocyte count helps to distinguish various types of anemia.[4] It is non-invasive, low cost, objective, early and reliable indicator of adequacy of response of a patient's bone marrow to erythropoietin (EPO) therapy in anemia of chronic disease (ACD) and blood conservation programme. In addition, Reticulocyte hemoglobin equivalent (RET-H_e) is used for measuring the hemoglobin content of reticulocytes, for diagnosing and monitoring cases of ACD especially in nephrological cases. It is also an early indicator of iron-restricted erythropoiesis in patients receiving erythropoietin therapy.[5,6]

Reticulocyte cellular indices such as MCVr (mean reticulocyte volume), CHr (reticulocyte hemoglobin content), CHCMr (reticulocyte hemoglobin concentration) generated by Advia® 2120i allows for assessment of the functional state of the erythropoiesis in the diagnosis and monitoring of recombinant human erythropoietin (rhEpo) therapy.[7]

Functional iron deficiency occurs in patients even in the presence of storage iron and/or oral iron supplementation occurs during intense stimulation by endogenous EPO response to anemia or to pharmacologic therapy with erythropoiesis-stimulating agents (ESAs). Iron sequestration mediated by hepcidin is an underappreciated and is the common cause of iron-restricted erythropoiesis in elderly patients with chronic inflammatory disease.[8]

MATERIAL AND METHOD

The index observational prospective study was conducted at a "Tertiary Care Super-Speciality Hospital" over a period of 18 months. The patterns of different conventional and newer hematological parameters in 40 patients of anemia having history of chronic kidney cases were studied. EDTA blood sample were processed through ACC like ADVIA® 2120i. PBS of each case was examined by two technicians followed by hematopathologist. Iron studies were advised on the basis of individual case. After erythropoietin therapy, the second EDTA samples were taken on 14th and 28th day of follow up. The association between hematological, morphological and clinical features were tested using Student's t-test, Fisher's exact test. All statistical results were analyzed by using SPSS 21.0 software considering p value <0.05 as significant.

RESULTS AND ANALYSIS

Anemia was diagnosed when the hemoglobin value is <12.0 g/dl for adult females, 13.0 g/dl for adult males, and 11.5g/dl for pediatric cases. Anemic patients with history of chronic renal diseases were selected among adults and children either admitted in our hospital or being followed up in OPD. All the parameters results of automated analyzers were microscopically checked by two experienced technologists using Leishman-Giemsa stained blood smears which were then confirmed by a hematopathologist.

Chr is an early measure of functional iron deficiency because reticulocytes are the earliest erythrocytes released into blood and circulate for 1-2 days. CHr was the first parameter that responded to iron therapy even before MCV and IRF. CHr rose upto normal value within two days while IRF takes around 10 days to recover. In CKD, erythropoietin therapy responsible for the increase in CHr, MCVr, IRF, and reticulocyte% in 21 to 28 days.

Parameters	Control (50)	IDA (40)	CKD (40)	Thalassemia (10)
MCV (fl)	85.2 ±4.6	71.3±48	82.9±6.1	67.8±4.1
MCVr (fl)	99.1±6.2	90.5±5.4	98.6±4.3	87.2±6
MCVr /MCV ratio	1.12	1.26	1.18	1.28
CHCM (g/dl)	40.2±1.9	29.4±2.1	29.9±2.4	31.6±1.1
CHCMr (g/dl)	31.7±1.4	23.4±3.2	23.7±2.2	24.4±1.6
CHCMr/CHCM ratio	0.79	0.79	0.79	0.77
CH (pg)	30.3±1.8	20.5±1.6	22.7±2.1	21.1±1.4
CHr (pg)	32.4±1.7	21.1±1.7*	24.8±1.6	21.7±1.7*
CH / CHr ratio	0.93	0.98	0.91	0.97
% Retic	1.19±0.15	0.34±0.06	0.66±0.08	1±0.15
IRF	5.2±0.6	0.62±0.1**	0.74±0.20	2.9±0.5**

DISCUSSION

The word 'Anemia' is derived from Greek word 'Anemia' (an=not, naime=blood) i.e. not having blood.[9] Immature reticulocyte fraction is an important parameter to identify ACD and nutritional anemia cases on basis of serum Iron studies. Serum iron levels are a direct measure of amount of iron bound to transferrin.[10] The levels are increased in hemosiderosis, hemolytic anemia, aplastic anemia, sideroblastic anemia, thalassemia, renal dialysis, multiple transfusions, and oral contraceptive use while decreased in ACD, acute inflammation, nephrosis, impaired iron absorption etc. TIBC is a useful index of nutritional status and discriminates well between people with biochemically defined IDA and ACD.[10] A. A low TIBC or transferrin may occur in hemochromatosis, ACD and malnutrition. There were directly proportional and indirectly proportional relationship of BM iron grade with serum ferritin and TIBC respectively, therefore these two parameters can be used to distinguish between IDA and ACD cases.[10,11]

In anemia due to reduced erythropoietin formation as in chronic kidney diseases, the reticulocyte count is an indicator of effective erythropoiesis. With the help of Advia^{R}2120i, more precise counting of reticulocytes and IRF is feasible. Our results of IRF as an early and sensitive index of marrow erythropoietic activity and hematopoietic recovery are similar to Łuczyński W et al.[12] CHr found to be more significant parameter than MCV, reticulocyte% and IRF in the diagnosis of IDA and is first parameter that responded to iron therapy in IDA as CHr rise upto normal value within 2 days while IRF takes around 10 to 14 days to recover. Our results of CHr as useful indicator for monitoring early response to intravenous iron therapy in IDA were in concordance with Buttarello M et al[13] CHr was decreased in IDA, CKD and thalassemia major group as compared to control and increased in vitamin B12 group and these results were similar to C. Ceylan.[14] The vitamin B12 deficiency group had significantly increased MCVr value as compared to control and other diseased group (p value <0.05). Among microcytic anemias, only IRF and reticulocyte% are significant between IDA and thalassemia major (p value <0.05). MCVr was significantly increased in vitamin B12 group. CH/CHr, MCVr/MCV, CHCMr/CHCM ratios cannot be used to distinguish IDA and thalassemia as it is not statistically significant (p value >0.05).

The complete reticulocyte picture, total reticulocyte, IRF, RET-H_e, will provide the clinicians with more comprehensive anemia workup of patients without access to primary care and less variation than acute phase reactants in patients with chronic renal diseases, inflammation or infection.

CONCLUSION

In case of CKD, IRF comes to normal level after 21-28 days of erythropoietin therapy. Moreover, it should be remembered that despite the essential role of automation, microscopy of pathologic samples remains essential. The relevance of analyzing newer parameters is not only cost effective, sensitive, specific but a faster mode in diagnosis and follow-up cases also.

Informed consent: A written consent in the language the patients understands was taken from all the subjects being enrolled after explaining the objectives and benefits of the study to them.

Ethical clearance: The study was then undertaken after due approval of the hospital ethics committee.

Funding resources: No funding was obtained from any external source.

ACKNOWLEDGEMENT: We acknowledge the support of our patients, as with their support the study had been feasible.

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